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60/404,922 22 August 2002 (22.08.2002) US
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- (74) Agent: McKAY, Margaret; National Research Council of Canada, Intellectual Property Services Office, EG-12, Bldg. M-58, 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*
- Published:
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: A GENOMIC APPROACH TO IDENTIFICATION OF NOVEL BROAD-SPECTRUM ANTIMICROBIAL PEPTIDES FROM BONY FISH

(57) Abstract: There is provided a method of identifying candidate nucleic acid sequences encoding antimicrobial peptides. The method comprises: identifying an initial peptide of interest; identifying genomic DNA encoding the initial peptide; identifying a flanking sequence on each side of the initial peptide; obtaining primers complementary to the flanking sequences; and, screening a wide range of nucleic acid sequences to identify candidate sequences capable of being amplified using the primers from step e). In some instances the antimicrobial peptide is a hepcidin or a pleurocidin.

WO 2004/018706 A2

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:

NATIONAL RESEARCH COUNCIL OF CANADA
Attn. McKay, Margaret
Intellectual Prop. Service Office
EG-12, Bldg. M-58, 1200 Montreal Rd.
Ottawa, Ontario K1A0R6
CANADA

Date of mailing
(day/month/year)

08/07/2004

Applicant's or agent's file reference

11402-98

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/CA 03/01323

International filing date
(day/month/year)

22/08/2003

Applicant

NATIONAL RESEARCH COUNCIL OF CANADA

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 48):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Catriona Cleere

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)".

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 11402-98	FOR FURTHER ACTION <small>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</small>	
International application No. PCT/CA 03/01323	International filing date (day/month/year) 22/08/2003	(Earliest) Priority Date (day/month/year) 22/08/2002
Applicant NATIONAL RESEARCH COUNCIL OF CANADA		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.
☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☒ Certain claims were found unsearchable (See Box I).

3. ☒ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/01323

CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47 A61K38/17 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DOUGLAS S E ET AL: "Cloning and developmental expression of a family of pleurocidin-like antimicrobial peptides from winter flounder, <i>Pleuronectes americanus</i> (Walbaum)" DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, vol. 25, no. 2, March 2000 (2000-03), pages 137-147, XP002273275 & ISSN: 0145-305X cited in the application abstract page 138, right-hand column, paragraph 2 - page 142, left-hand column, paragraph 1 figures 1,3,4 table 1</p> <p style="text-align: center;">-/-</p>	1-7, 11, 12, 18

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

24 March 2004

Date of mailing of the International search report

08. 07. 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Ulbrecht, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/01323

(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DOUGLAS SUSAN E ET AL: "Identification and expression analysis of hepcidin-like antimicrobial peptides in bony fish." DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, vol. 27, no. 6-7, July 2003 (2003-07), pages 589-601, XP002273276 ISSN: 0145-305X abstract page 590, right-hand column, paragraph 3 - page 595, right-hand column, paragraph 1 figures 1-3 table 1	1-7,11, 12,18
A	COLE ALEXANDER M ET AL: "Characterization of a fish antimicrobial peptide: Gene expression, subcellular localization, and spectrum of activity" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 44, no. 8, August 2000 (2000-08), pages 2039-2045, XP002273277 & ISSN: 0066-4804 cited in the application abstract page 2039, left-hand column, paragraph 2 - page 2040, right-hand column, paragraph 6 figures 1,2	1-7,11, 12,18
A	COLE A M ET AL: "ISOLATION AND CHARACTERIZATION OF PLEUROCIDIN, AN ANTIMICROBIAL PEPTIDE IN THE SKIN SECRETIONS OF WINTER FLOUNDER" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 18, 2 May 1997 (1997-05-02), pages 12008-12013, XP000919411 ISSN: 0021-9258 abstract page 12008, right-hand column, paragraph 5 - page 12010, left-hand column, paragraph 3 figure 2	1-7,11, 12,18
A	US 6 288 212 B1 (JIA XIAOYAN ET AL) 11 September 2001 (2001-09-11) example 6 table 10	1-7,11, 12,18

-/-

INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCT/CA 03/01323

(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHIKE HIROKO ET AL: "Bass hepcidin is a novel antimicrobial peptide induced by bacterial challenge" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 269, no. 8, April 2002 (2002-04), pages 2232-2237, XP002273278 & ISSN: 0014-2956 cited in the application the whole document	1-7, 11, 12, 18
A	-& DATABASE GENBANK 'Online! NCBI; 22 August 2001 (2001-08-22), MARTIN SA: "EST00598" XP002273280 retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. BI468191 the whole document	1-7, 11, 12, 18
A	-& DATABASE GENBANK 'Online! NCBI; 9 September 1999 (1999-09-09), REITH M: "Ldt-0110" XP002273281 retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. AW013026 the whole document	1-7, 11, 12, 18
A	-& DATABASE GENBANK 'Online! NCBI; 11 May 2000 (2000-05-11), GRACEY: "4268263" XP002273367 retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. AW783824 the whole document	1-7, 11, 12, 18
A	BAYNE C J ET AL: "Immune-relevant (including acute phase) genes identified in the livers of rainbow trout, Oncorhynchus mykiss, by means of suppression subtractive hybridization" DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, vol. 25, no. 3, April 2001 (2001-04), pages 205-217, XP002273279 & ISSN: 0145-305X page 206, right-hand column, paragraph 2 - page 207, left-hand column, paragraph 2 figure 4	1-7, 11, 12, 18
A	& DATABASE GENBANK 'Online! NCBI; 20 February 2002 (2002-02-20), BAYNE ET AL.: "Oncorhynchus mykiss putative hepcidin antibacterial peptide mRNA" retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. AF281354 the whole document	1-7, 11, 12, 18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 03/01323

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6288212	B1	11-09-2001	AU 758698 B2 27-03-2003
			AU 5789099 A 21-03-2000
			CA 2341340 A1 09-03-2000
			EP 1107976 A1 20-06-2001
			WO 0012528 A1 09-03-2000
			US 2003096949 A1 22-05-2003